

The following Listing of the Claims will replace all prior versions and all prior listings of the claims in the present application:

Listing of The Claims:

1. (Currently Allowed) A purified thermostable DNA polymerase having an amino acid sequence presented in SEQ ID NO: 2 from residue 1 to 776.
2. (Currently Cancelled) The polymerase of claim 1 that is isolated from *Thermococcus* species JDF-3.
3. (Currently Cancelled) The polymerase of claim 1 that is isolated from a recombinant organism transformed with a vector that codes for the expression of *Thermococcus* species JDF-3 DNA polymerase.
4. (Previously Cancelled)
- BS 5. (Currently Allowed) An isolated recombinant polypeptide comprising the amino acid sequence of SEQ ID NO: 2.
6. (Currently Cancelled) An isolated recombinant mutant of the *Thermococcus* JDF-3 Family B DNA polymerase of SEQ ID NO: 2 that is 3' to 5' exonuclease deficient.
7. (Currently Cancelled) The isolated recombinant DNA polymerase of claim 6, wherein said polymerase has a dual mutation comprising a serine to proline mutation at a site corresponding to S345 of SEQ ID NO: 2; and a proline to leucine mutation at a site corresponding to P410 of SEQ ID NO: 2.
8. (Currently Cancelled) The isolated recombinant DNA polymerase of claim 6 that has an aspartic acid to threonine or alanine mutation at the amino acid corresponding to D141 of SEQ ID NO: 2 or a glutamic acid to alanine mutation at the amino acid corresponding to E143 of SEQ ID NO: 2.

9. (Currently Cancelled) The isolated recombinant DNA polymerase of claim 6 that has an aspartic acid to threonine or alanine mutation at the amino acid corresponding to D141 of SEQ ID NO: 2 and a glutamic acid to alanine mutation at the amino acid corresponding to E143 of SEQ ID NO: 2.

10. (Currently Cancelled) An isolated recombinant Family B DNA polymerase having reduced discrimination against non-conventional nucleotides, wherein said DNA polymerase has a mutation in the Region II consensus sequence DXXSLYPSII.

11. (Previously Cancelled).

ES 12. (Currently Cancelled) The DNA polymerase of claim 6 wherein said DNA polymerase further comprises a mutation selected from the group consisting of: a leucine to histidine mutation at a site corresponding to L408 of SEQ ID NO: 2; a leucine to phenylalanine mutation at a site corresponding to L408 of SEQ ID NO: 2; a proline to leucine mutation at a site corresponding to P410 of SEQ ID NO: 2; and an alanine to threonine mutation at a site corresponding to A485 of SEQ ID NO: 2.

13. (Currently Cancelled) The DNA polymerase of claim 12 wherein said polymerase having said alanine to threonine mutation at said site corresponding to A485 of SEQ ID NO:2 further comprises a mutation selected from the group consisting of: a leucine to histidine mutation at a site corresponding to L408 of SEQ ID NO: 2; a leucine to phenylalanine mutation at a site corresponding to L408 of SEQ ID NO: 2; a serine to proline mutation at a site corresponding to S345 of SEQ ID NO: 2; and a proline to leucine mutation at a site corresponding to P410 of SEQ ID NO: 2.

14. (Currently Cancelled) The DNA polymerase of claim 6 that has reduced discrimination against a non-conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides and conjugated nucleotides.

15. (Currently Cancelled) The DNA polymerase of claim 14 wherein said conjugated nucleotide is selected from the group consisting of radiolabeled nucleotides, fluorescently labeled

nucleotides, biotin labeled nucleotides, chemiluminescently labeled nucleotides and quantum dot labeled nucleotides.

16. (Currently Cancelled) An isolated recombinant Family B DNA polymerase comprising an alanine to threonine mutation at the site corresponding to A485 of SEQ ID NO: 2, or a mutation at a site corresponding to L408, S345 or P410 of SEQ ID NO: 2, wherein said DNA polymerase has reduced discrimination against non-conventional nucleotides relative to the wild-type form of that polymerase.

17. (Currently Cancelled) The polymerase of claim 16 that is 3' to 5' exonuclease deficient.

18. (Currently Cancelled) The polymerase of claim 17 that has a mutation at an amino acid corresponding to D141 or E143 of SEQ ID NO: 2.

19. (Currently Cancelled) The polymerase of claim 17 that has an aspartic acid to threonine or alanine mutation at a site corresponding to D141 of SEQ ID NO: 2.

ES 20. (Currently Cancelled) The polymerase of claim 17 that has a glutamic acid to alanine mutation at a site corresponding to E143 of SEQ ID NO: 2.

21. (Currently Cancelled) The polymerase of claim 20 that has an aspartic acid to threonine or alanine mutation at the amino acid corresponding to D141 of SEQ ID NO: 2.

22. (Currently Cancelled) The polymerase of claim 16 that is thermostable.

23. (Currently Cancelled) The polymerase of claim 16 that is archaeal.

24. (Currently Cancelled) The polymerase of claim 16 wherein said DNA polymerase comprises a leucine to histidine mutation at a site corresponding to L408 of SEQ ID NO: 2.

25. (Currently Cancelled) The polymerase of claim 16 wherein said DNA polymerase comprises a leucine to phenylalanine mutation at a site corresponding to L408 of SEQ ID NO: 2.

26. (Currently Cancelled) The polymerase of claim 16 wherein said DNA polymerase comprises a proline to leucine mutation at a site corresponding to P410 of SEQ ID NO: 2.

27. (Currently Cancelled) The polymerase of claim 16 wherein said DNA polymerase comprises a serine to proline mutation at a site corresponding to S345 of SEQ ID NO: 2, wherein said polymerase may further comprise a mutation at a site corresponding to T604 of SEQ ID NO: 2.

28. (Currently Cancelled) The polymerase of claim 16, wherein said DNA polymerase comprises a tyrosine to cysteine mutation at a site corresponding to Y497 of SEQ ID NO: 2, wherein said polymerase may further comprise an isoleucine to valine mutation at a site corresponding to I630 of SEQ ID NO: 2.

29. (Currently Cancelled) The polymerase of claim 16, wherein said DNA polymerase comprises a glutamic acid to lysine mutation at a site corresponding to E645 of SEQ ID NO: 2.

25 30. (Currently Cancelled) The polymerase of claim 16, wherein said DNA polymerase comprises a glutamic acid to lysine mutation at a site corresponding to E578 of SEQ ID NO: 2, wherein said polymerase may further comprise an arginine to methionine mutation at a site corresponding to R465 of SEQ ID NO: 2.

31. (Currently Cancelled) The polymerase of claim 16, wherein said DNA polymerase comprises a leucine to glutamine mutation at a site corresponding to L396 of SEQ ID NO: 2, wherein said polymerase further comprises a mutation at a site corresponding to V401, N424, P569, E617, or V640 of SEQ ID NO: 2.

32. (Currently Cancelled) The polymerase of claim 16, wherein said DNA polymerase comprises a serine to asparagine mutation at a site corresponding to S651 of SEQ ID NO: 2.

33. (Currently Cancelled) The polymerase of claim 16, wherein said DNA polymerase comprises a leucine to proline mutation at a site corresponding to L396 of SEQ ID NO: 2, wherein said polymerase may further comprise a mutation at a site corresponding to E459 of SEQ ID NO: 2.

34. (Currently Cancelled) The polymerase of claim 16, wherein said DNA polymerase comprises a leucine to proline mutation at a site corresponding to L456 of SEQ ID NO: 2, wherein said polymerase may further comprise a mutation at a site corresponding to E658 of SEQ ID NO: 2.

35. (Currently Cancelled) The polymerase of claim 16, wherein said DNA polymerase comprises a leucine to histidine mutation at a site corresponding to L408 of SEQ ID NO: 2, wherein said polymerase may further comprise a mutation at a site corresponding to V437, or L478 of SEQ ID NO: 2.

36. (Currently Cancelled) The polymerase of claim 16 wherein said DNA polymerase comprises an tyrosine to asparagine mutation at a site corresponding to Y496 of SEQ ID NO: 2.

37. (Currently Cancelled) The polymerase of claim 16 wherein said DNA polymerase comprises an alanine to threonine mutation at a site corresponding to A485 of SEQ ID NO: 2.

B5 38. (Currently Cancelled) The polymerase of claim 37 comprising a leucine to histidine mutation at a site corresponding to L408 of SEQ ID NO: 2.

39. (Currently Cancelled) The polymerase of claim 37 comprising a leucine to phenylalanine mutation at a site corresponding to L408 of SEQ ID NO: 2.

40. (Currently Cancelled) The polymerase of claim 37 comprising a proline to leucine mutation at a site corresponding to P410 of SEQ ID NO: 2.

41. (Currently Cancelled) The polymerase of claim 16, having reduced discrimination against a non-conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides and conjugated nucleotides.

42. (Currently Cancelled) The polymerase of claim 41 wherein said conjugated nucleotide is selected from the group consisting of radiolabeled nucleotides, fluorescently labeled nucleotides,

biotin labeled nucleotides, chemiluminescently labeled nucleotides and quantum dot labeled nucleotides.

43. (Currently Cancelled) The polymerase of claim 10 or 16 further comprising a mutation at an amino acid residue in the polymerase that corresponds to a mutation selected from the group consisting of: a Y to V mutation at amino acid 409 of SEQ ID NO:2; an A to C, S, L, I, F, or V mutation at amino acid 485 of SEQ ID NO: 2; a Y to S mutation at amino acid 494 of SEQ ID NO: 2; a Y to L mutation at amino acid 496 of SEQ ID NO: 2; and an A to Y mutation at amino acid 490 of SEQ ID NO: 2.

44. (Currently Cancelled) The polymerase of claim 10 or 16 further comprising a mutation at an amino acid of the polymerase corresponding to one of amino acids 483 to 496, inclusive, of SEQ ID NO: 2.

E5 45. (Currently Cancelled) The polymerase of claim 44 wherein said mutation is at an amino acid of the polymerase corresponding to one of amino acids 485, 490, 494, or 496 of SEQ ID NO: 2.

46. (Currently Cancelled) An isolated recombinant Family B DNA polymerase comprising an alanine to threonine mutation at an amino acid corresponding to A485T of SEQ ID NO: 2 and and at least one substitution in the polymerase of an amino acid corresponding to L408, Y409, S345 or P410 respectively, of SEQ ID NO: 2.

47. (Currently Cancelled) An isolated recombinant Family B DNA polymerase comprising an amino acid other than A at an amino acid of the polymerase corresponding to A485 of SEQ ID NO: 2, and at least one substitution in the polymerase of an amino acid corresponding to L408, Y409, S345 or P410, respectively, of SEQ ID NO: 2.

85. (Currently Cancelled) The DNA polymerase of claim 10 wherein said mutation in Region II is selected from the group consisting of: a leucine to histidine mutation at a site corresponding to L408 of SEQ ID NO: 2; a leucine to phenylalanine mutation at a site corresponding to L408 of SEQ ID NO: 2; a proline to leucine mutation at a site corresponding to P410 of SEQ ID NO: 2.

86. (Currently Cancelled) The DNA polymerase of claim 10, said polymerase further comprising an alanine to threonine mutation at a site corresponding to A485 of SEQ ID NO: 2.

87. (Currently Cancelled) The DNA polymerase of claim 10 that has reduced discrimination against a non-conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides and conjugated nucleotides.

88. (Currently Cancelled) An isolated recombinant Family B DNA polymerase from *Thermococcus* species JDF-3 that comprises an alanine to threonine mutation at a site corresponding to A485 of SEQ ID NO: 2.

89. (Currently added) An isolated recombinant DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising a mutation at one or more amino acids in exo I (DXE) motif within said sequence of SEQ ID NO: 2.

85 90. (Currently added) An isolated recombinant DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising a mutation at one or more amino acids in exo I (DXE) motif within said sequence of SEQ ID NO: 2 and wherein said mutation in exo I (DXE) motif is selected from the group consisting of: aspartic acid (D) to threonine (T), aspartic acid (D) to alanine (A) and glutamic acid (E) to alanine (A).

91. (Currently added) An isolated recombinant DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising a mutation at one or more amino acids in exo I (DXE) motif within said sequence of SEQ ID NO: 2 and a mutation at one or more amino acids in Region II (DXXSLYPSII) within said sequence of SEQ ID NO: 2.

92. (Currently added) An isolated recombinant DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising a mutation at one or more amino acids in exo I (DXE) motif within said sequence of SEQ ID NO: 2 and a mutation at one or more amino acids in Region II (DXXSLYPSII) within said sequence of SEQ ID NO: 2, wherein said mutation in exo I (DXE) motif is selected from the group consisting of: aspartic acid (D) to threonine (T), aspartic acid (D) to alanine (A) and glutamic acid (E) to alanine (A), and said mutation in Region II

(DXXSLYPSII) is selected from the group consisting of: leucine (L) to histidine (H), leucine (L) to phenylalanine (F), and praline (P) to leucine (L).

93. (Currently added) The isolated recombinant DNA polymerase of any one of claims 89-92, further comprising a mutation at one or more additional amino acids selected from the group consisting of: S345, A485, T604, Y497, I630, E645, E578, R465, V401, N424, P569, E617, V640, S651, L396, E459, L456, E658, V437, L478, Y496, Y409 and Y490 within the sequence of SEQ ID NO: 2.

ES 94. (Currently added) The isolated recombinant polymerase of claim 93, wherein said mutation at S345 is serine (S) to proline (P), said mutation at A485 is alanine (A) to threonine (T), cysteine (C), serine (S), leucine (L), isoleucine (I), phenylalanine(F) or valine (V), said mutation at Y497 is tyrosine (Y) to cysteine (C), said mutation at I630 is isoleucine (I) to valine (V), said mutation at E645 is glutamic acid (E) to lysine (L), said mutation at E578 is glutamic acid (E) to lysine (L), said mutation at R465 is arginine (R) to methionine (M), said mutation at L396 is leucine (L) to glutamine (Q) or proline (P), said mutation at S651 is serine (S) to asparagine (B), said mutation at L456 is leucine (L) to histidine (H), said mutation at Y496 is tyrosine (Y) to asparagine (B) or leucine (L), said mutation at Y409 is tyrosine (Y) to valine (V), said mutation at Y490 is alanine (A) to tyrosine (Y).

95. (Currently added) The isolated recombinant DNA polymerase of any one of claims 89-91, wherein said isolated recombinant DNA polymerase has reduced discrimination against a non-conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides and conjugated nucleotides.

96. (Currently added) An isolated recombinant DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising a mutation at one or more amino acids in exo II (NX₂-₃FD) motif or exo III (YX₃D) within said sequence of SEQ ID NO: 2.

97. (Currently added) An isolated recombinant DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising a mutation at one or more amino acids in exo II (NX₂-

₃FD) motif or exo III (YX₃D) motif within said sequence of SEQ ID NO: 2 and a mutation at one or more amino acids in Region II (DXXSLYPSII) within said sequence of SEQ ID NO: 2.

98. (Currently added) The isolated recombinant DNA polymerase of claim 96 or 97, wherein said isolated recombinant DNA polymerase has reduced discrimination against a non-conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides and conjugated nucleotides.

99. (Currently added) An isolated recombinant family B DNA polymerase comprising a sequence selected from the sequences as indicated by accession numbers listed in Table II, and further comprising a mutation at one or more amino acids in exo I (DXE) motif within said sequence and a mutation at the leucine and/or proline positions in Region II (DXXSLYPSII) within said sequence.

ES 100. (Currently added) An isolated recombinant family B DNA polymerase comprising a sequence selected from the sequences listed in Table II as indicated by accession number, and further comprising a mutation at one or more amino acids in exo II (NX₂₋₃FD) motif or exo III (YX₃D) motif within said sequence and a mutation at the leucine and/or proline positions in Region II (DXXSLYPSII) within said sequence.

101. (Currently added) An isolated recombinant family B DNA polymerase comprising a sequence selected from the sequences as indicated by accession numbers listed in Table II, and further comprising a mutation at one or more amino acids in exo I (DXE) motif within said sequence, a mutation in Region II (DXXSLYPSII) within said sequence, and a mutation at amino acid corresponding to A485 of SEQ ID NO: 2.

102. (Currently added) An isolated recombinant family B DNA polymerase comprising a sequence selected from the sequences listed in Table II as indicated by accession number, and further comprising a mutation at one or more amino acids in exo II (NX₂₋₃FD) motif or exo III (YX₃D) motif within said sequence, a mutation in Region II (DXXSLYPSII) within said sequence, and a mutation at amino acid corresponding to A485 of SEQ ID NO: 2.

103. (Currently added) The isolated recombinant family B DNA polymerase of claim 99 or 100, further having reduced discrimination against a non-conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides and conjugated nucleotides.

104. (Currently added) An isolated recombinant thermostable family B DNA polymerase comprising a sequence selected from the sequences as indicated by accession numbers listed in Table II, and further comprising a mutation at one or more amino acids in exo I (DXE) motif within said sequence and a mutation at the leucine and/or proline positions in Region II (DXXSLYPSII) within said sequence.

ES 105. (Currently added) An isolated recombinant thermostable family B DNA polymerase comprising a sequence selected from the sequences as indicated by accession numbers listed in Table II, and further comprising a mutation at one or more amino acids in exo II (NX₂₋₃FD) motif or exo III (YX₃D) motif within said sequence and a mutation at the leucine and/or proline positions in Region II (DXXSLYPSII) within said sequence.

106. (Currently added) The isolated recombinant thermostable family B DNA polymerase of claim 104 or 105, further having reduced discrimination against a non-conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides and conjugated nucleotides.

107. (Currently added) The isolated recombinant thermostable family B DNA polymerase of claim 104 or 105, wherein said recombinant thermostable family B DNA polymerase is an archaeal DNA polymerase.

108. (Currently added) An isolated recombinant JDF-3 DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising a mutation at D141 and/or E143 within said sequence of SEQ ID NO: 2.

109. (Currently added) The isolated recombinant JDF-3 DNA polymerase of claim 108, wherein said mutation at D141 is an aspartic acid (D) to threonine (T) or alanine (A) mutation, and said mutation at E143 is a glutamic acid (E) to alanine (A) mutation.

110. (Currently added) The isolated recombinant JDF-3 DNA polymerase of any one of claims 108-109, further comprising a mutation at one or more amino acids of Region II (DXXSLYPSII) within said SEQ ID NO: 2.

111. (Currently added) The isolated recombinant JDF-3 DNA polymerase of claim 110, wherein said mutation at one or more amino acids is at L408 or P410 within Region II.

112. (Currently added) The isolated recombinant JDF-3 DNA polymerase of claim 111, wherein said mutation at one or more amino acids is selected from the group consisting of: a leucine (L) to histidine (H) or phenylalanine (F) mutation at L408 and a proline (P) to leucine (P) mutation at P410.

ES 113. (Currently added) The isolated recombinant JDF-3 DNA polymerase of any one of claims 108-109, further comprising a mutation at one or more additional amino acids selected from the group consisting of: A485, S345, T604, Y497, I630, E645, E578, R465, V401, N424, P569, E617, V640, S651, L396, E459, L456, E658, V437, L478, Y496, Y409 and Y490 within the sequence of SEC ID NO: 2.

114. (Currently added) The isolated recombinant LDF-3 DNA polymerase of claim 108, wherein said mutation at S345 is serine (S) to proline (P), said mutation at A485 is alanine (A) to threonine (T), cysteine (C), serine (S), leucine (L), isoleucine (I), phenylalanine(F) or valine (V), said mutation at Y497 is tyrosine (Y) to cysteine (C), said mutation at I630 is isoleucine (I) to valine (V), said mutation at E645 is glutamic acid (E) to lysine (L), said mutation at E578 is glutamic acid (E) to lysine (L), said mutation at R465 is arginine (R) to methionine (M), said mutation at L396 is leucine (L) to glutamine (Q) or to proline (P), said mutation at S651 is serine (S) to asparagine (B), said mutation at L456 is leucine (L) to histidine (H), said mutation at Y496 is tyrosine (Y) to asparagine (B) or leucine (L), said mutation at Y409 is tyrosine (Y) to valine (V), said mutation at Y490 is alanine (A) to tyrosine (Y).

115. (Currently added) The isolated recombinant JDF-3 DNA polymerase of any one of claims 108-109, wherein said JDF-3 DNA polymerase has reduced discrimination against a non-

conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides and conjugated nucleotides.

116. (Currently added) The isolated recombinant JDF-3 DNA polymerase of claim 115, wherein said conjugated nucleotide is selected from the group consisting of radiolabeled nucleotides, fluorescently labeled nucleotides, biotin labeled nucleotides, chemiluminescently labeled nucleotides and quantum dot labeled nucleotides.

117. (Currently added) An isolated JDF-3 DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising the following mutations: D141T or D141A, E143A, L408H or L408F, P410L, and A485T within said sequence ID NO: 2.

118. (Currently added) An isolated JDF-3 DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising the following mutations: D141T or D141A and E143A within said sequence ID NO: 2.

119. (Currently added) An isolated JDF-3 DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising the following mutations: D141T or D141A and E143A, and further comprising one or more mutations selected from the group consisting of: L408H or L408F, P410L, and S345P within said sequence ID NO: 2.

120. (Currently added) An isolated JDF-3 DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising mutations at: D141, E143, P410, and A485 within said sequence ID NO: 2.

121. (Currently added) An isolated JDF-3 DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising the following mutations of: D141T or D141A, E143A, P410L, and A485T within said sequence ID NO: 2.

122. (Currently added) A kit comprising an isolated recombinant DNA polymerase of any one of claims 89-92, and packaging material thereof.

123. (Currently added) A kit comprising an isolated recombinant DNA polymerase of any one of claims 96-97, and packaging material thereof.

124. (Currently added) A kit comprising an isolated recombinant DNA polymerase of any one of claims 99-102, and packaging material thereof.

125. (Currently added) A kit comprising an isolated recombinant DNA polymerase of any one of claims 104-105, and packaging material thereof.

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126. (Currently added) A kit comprising an isolated recombinant DNA polymerase of any one of claims 108, 117-119, and packaging material thereof.

127. (Currently added) A kit comprising an isolated recombinant DNA polymerase of any one of claims 120-121, and packaging material thereof.
